

RESEARCH ARTICLE

## Evaluation of Antibacterial Activity in Nanoparticles Ointment Preparation using Ethanol Extract of Miana Leaves (*Coleus Atropurpureus (L.) Benth*)

Tria Prayoga\*

Department of Pharmacy, IKIFA Pharmacy Academy, East Jakarta, Indonesia

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### ABSTRACT

**Objective(s):** Compared the nanoparticles form of Miana leaves with its extracts, even in single state or ointment preparation. Hence, SPSS program using Paired T Test is obtained to know the differences between them.

**Methods:** The method used in this research is experiment in which try to change the extract form to be nanoparticles and be compared. The mixture of Poly Acrylic Acid (PAA) and calcium chloride using ionic glass method could change the size of Miana leaves' ethanol extract to be nanoparticle with the average diameter amounted 89,77 nm. The equality showed that 2,50% of Miana leaves is equal with 1,17% of nanoparticles Miana leaves extract.

**Results:** The result indicated that ethanol Miana leaves extract had antibacterial activities toward *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The nanoparticles form proven can improve the stability and medicine's administration. In the medicine administration system, nanoparticles has function as carrier by dissolving, trap, encapsulate, and attached the medicine in the matrix. Then, by mixed poly acrylic acid and calcium chloride using ionic glass method, the nanoparticles would be formed.

**Conclusions:** This research indicated that Miana leaves extract of nanoparticles ointment is safe and did not irritate the skin.

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## INTRODUCTION

The prevalence of infection disease is still quite high due to the high cost in its treatment and medication. Thus, it has led some experts to invest their knowledge in formulating herbal medication for infection disease in which all elements of society can use the medicine [1]. In this case, the development of antimicrobial medicine depicted as an important key of advancement in the medication field since it is effective to treat infection, improve life quality and medical field as well as pharmacy industries [2].

Nowadays, there are a lot of traditional medication that used by society, yet it does not support by research studies. Therefore, it is needed to conduct research aimed to know the beneficial of traditional medicine [3]. Miana (*Coleus atropurpureus* [L.]

Benth) is described as ornamental plants which can be used as traditional medicine comes from Southeast Asia. The shape and color of Miana leaves are varied, and one of them which have medicine beneficial is the brownish red leaves [4]. Miana leaves has chemical compound as like flavonoid, polyphenol, tannin, saponin, alkaloid, caffeic acid, rosmarinic acid, p-coumaric acid, gallic acid, quercetin, ethyl acetate, methyl eugenol, eugenol, thymol, phenol, carvacrol, phytosterols, mineral, and essential oil [5, 6, 7]. Generally, Miana leaves consume empirically by society by boil the leaves and is used for drink. In fact, Miana leaves have many beneficial such as for boil, ear and eyes inflammation medication, meanwhile its root can be used for diarrhea and stomach ache medication [8, 9].

\* Corresponding Author Email: [trayoga66@gmail.com](mailto:trayoga66@gmail.com)

Flavonoids were usually synthesized by plants in reaction to the microbial infection, so therefore it is common if flavonoid were effective antimicrobial substances on various types of microorganisms by *in vitro*. Moreover, it has been reported that other species were having antibacterial activities [10, 11, 12, 13]. Several kinds of flavonoids that have antibacterial activity are apigenin, galangin, flavon, flavonoid glycoside, isoflavon, flavanon, and chalcones [14].

Based on the previous studies, Miana leaves' ethanol extract is having the antibacterial activities toward, *E. coli*, *S. aureus* and *P. aeruginosa*. Furthermore, it can be used to heal infected wound in rabbit in a form of nanoparticles in 40% concentration [15, 16].

Hence, this research aimed to know whether nanoparticle form of Miana leaves of ethanol extract is better than the extracts, even in single or ointment preparation toward *E. coli* and *S. aureus*.

## MATERIAL AND METHODS

### Materials

The plant used in this research is Miana leaves (*Coleus scutellarioides* [L.] Benth) which are generated from Laboratory of Plants, Spices, and Medicine, Bogor, Indonesia. Afterward, for plant taxonomy is conducted in the Central Plants Conservation, Bogor Botanical Gardens, Indonesian Institute of Sciences, Bogor.

Besides Miana leaves, there are several materials used such as distilled water; Poly Acrylic Acid (Sigma Aldrich); Calcium Chloride (Merck); Avicel PH 102 (Bratachem); Chloride Acid (Merck); Natrium Hydroxide (Merck); Nutrient agar (Merck); Pepton Broth (Merck); *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 29322; Chloramphenicol (Bratachem); Liquid Paraffin (Bratachem); Methyl Paraben (Merck); Propyl Paraben (Merck); Alcohol (Bratachem).

In addition, the tools which are used including Homogenizer (Hsiangtai); Spread ability test tools; analytic scales (AND GR-200); Inoculating Loop; Alcohol Lamp; Laminar Air Flow (Gelman Sciences); Spectrophotometer UV-Vis; Autoclave (Hirayana); Oven (Memmert); Petri Dish; Test Tube; 9Incubator (Memmert); Vortex (Thermolyne); Brookfield Viscometer (DV-II+Pro); Particle Size Analyser (DelsaNano™); Spray Dryer (Buchi Spray-dryer B-190); pH meter (PHM201); Microscope Slide; Stirrer; Desiccator;

Magnetic Stirrer (Cimarec-2); Caliper (TRICLE); Micropipette; Waterbath; Silica gel 60 F254 nm.

### Methods

#### Miana Leaves Extraction

Miana leaves which have dried are being powdered with a blender. Then, it is sifted with number 60 sieve, and is extracted with maceration method. Afterward, 250 gram of Miana leaves in a form of simplicia powder is put in jar, soaked with 96% ethanol dissolve amounted 900 ml along 24 hours and filtered. The left pulp then is added with 96% ethanol dissolve amounted 300 ml. Maceration method is repeated for three times so that the total amount will be 1,8 liter for a jar and the time for maceration was 4 days. In this research, the jar used were 8 jars, so that the total will be 14,4 liter. All of filtrate then is concentrated with rotary evaporator vacuum.

#### Extract Characterization/Organoleptic Testing

Organoleptic testing or extract characterization is including into specific parameter aims to describe shape, color, smell, and flavor which can be determined by sensing. Moreover, it aims as first introductory toward subjective extract.

#### Nanoparticle Preparation of Miana Leaves Extract using PAA and Calcium Chloride

A number of Miana leaves is dissolved using suitable solvent, then added 0,1% PAA amounted 20 ml and distilled water up to 40 ml. It is stirred using magnetic stirrer with 400 rpm speed for 3 minutes. Afterward, pH adjust is obtained by adding natrium hydroxide until the pH reach number 8. If the pH has reached more than 8, it is needed to add chloride acid and drop calcium chloride until the turbidity formed.

#### Nanoparticles Characterization

Nanoparticle characterization is involving the observation of size and Miana leaves extract. Particle size analyzer is used as a tool.

#### Ointment Preparation using Miana Leaves Extract

The ointment base that used in this research is hydrocarbon base [17]. Afterward, liquid paraffin is mixed with white Vaseline and stirred till the Vaseline melted (M1). Then, propyl and methyl paraben is dissolved using 96% alcohol and Miana leaves extract is added (M2). Once if M1 become cool, M2 is mixed using homogenizer.

*Antibacterial Activities Assay*

100 µL bacterial suspensions is added into 20 ml of sterile nutrient agar then rotated to be homogeneous. In case to make the bacterial suspension equal, hence it is poured into petri dish and rotated to the right and left. When the agar is become condensed, 4 holes was made using drill. Afterward, the Miana leaves extract, nanoparticles ointment using Miana leaves extract, chloramphenicol positive control 4 mg/ml in ethanol as well as negative control of ointment were poured into holes. It was obtained aseptically in laminar air flow, incubated in 35°-37°C for 24 hours. The pellucid area around holes shows that there was bacterial inhibition zone and measured using caliper.

*Statistical Analysis*

The data of Miana leaves extract activities, nanoparticles of Miana leaves extract activities, and evaluation of ointment preparation including viscosity, spread ability, pH, and ointment preparation activities were evaluated its normality since it requirement for parametric testing. If the data have been stated as normal, hence paired sample t-test was conducted since there was similarity in samples. By using paired sample t-test, Miana leaves can be compared with the form of nanoparticles. The statistical analysis is also obtained to know the difference significant between Miana leaves extract with nanoparticles

Miana leaves extract using 0,05 significant value.

*Decision taking:*

a. If the significant value (2-tailed) < 0,05, so that there were difference significant between Miana leaves extract and nanoparticles using Miana leaves extract.

b. If the significant value (2-tailed) > 0,05, so that there were no difference between Miana leaves extract and nanoparticles using Miana leaves extract.

**RESULTS**

*Miana leaves Extract Preparation*

2 kg of Miana leaves is macerated with 90% of ethanol dissolve, the total of dissolve used amounted 14,4 liter. Furthermore, the concentrated extract that is obtained after being concentrated is about 210,97 gram with a yield amounted 10,54% and DER-native 9,48%.

The yield counted to know how much the percentage of the extract in simplicia powder. Meanwhile, DER-native counted to know how much (i.e gram) of simplicia powder needed to produce 1 gram of extract.

*Organoleptic Extract Assays*

The result of organoleptic extract assays can be seen in Table 1 below. The result showed that the shape of extract is thick, has black color, aromatic smell, and has bitter flavor observed through sensing.

Tabel 1. Result of Miana leaves extract observation

No.	Organoleptic	Result of Observation
1.	Shape	Extra Thick
2.	Color	Black
3.	Smell	Aromatic
4.	Flavor	Bitter

Table 2. the result observation of nanoparticles using Miana leaves extract

No.	Parameter	Day 1	Day 2	Day 3	Day 4	Day 5
1.	Colour	Light brown				
2.	Turbidity	Stable	Stable	Stable	Stable	Stable
3.	Sediment	None	None	None	None	None

Table 3. The result of nanoparticles size and distribution

Number.	Repetition	Average Diameter (nm)	Particle Size Distribution		
			10%	50%	90%
1.	1	86	67	75,9	105,2
2.	2	117,7	91,5	103,8	145
3.	3	65,6	50,9	57,8	80,7
4.	Average	89,77	69,8	79,17	110,3



*Preparation of Nanoparticles Miana Leaves Extract using PAA and Calcium Chloride*

100 gram of Miana leaves extract is being added by 96% alcohol amounted 15 ml, 0,5% PAA amounted 5 ml, propylene glycol amounted 15 ml and distilled water up to 50 ml. Then, it is stirred using magnetic stirrer with 400 rpm speed for 3 minutes. The pH is adjusted by adding natrium hydroxide until reach pH 8, but if the pH was more than 8, the chloride acid will be added. Calcium chloride is dropped until the turbidity become homogenous. However, for about 15 minutes, the nanoparticles formed have no changes. Here, Table 2 demonstrates the result observation of nanoparticles using Miana leaves extract dissolve along 5 days including color, turbidity, and sediment.

*Nanoparticles Characterization Assays*

According to the measurement using particle size analyzer DelsaNano™, it can be concluded that Miana leaves extract has fulfilled the nanoparticles requirement since it still on range 10-1000 nm amounted 89,77 nm.

*The Formulation of Antibacterial Ointment contained Nanoparticles using Miana Leaves Extract*

The equivalence value for 2,5% Miana leaves extract is about 1,17% nanoparticles using Miana leaves extract powder, thus the formula obtained based on Table 4.

*Anti-bacterial Activity Testing*

The inhibition diameter evaluation is obtained in purpose to know the concentration that can

prevent the bacteria growth of *E. coli* and *S. aureus*. In the concentration 20, 40, and 80%, there is clear zone in the agar media which contains *S. aureus* and *E. coli* bacteria. Hence, Miana leaves extract have antibacterial activity toward *E.coli* and *S. aureus*.

Based on the table above, it can be concluded that the concentration increasing of Miana leaves extract influences the clear zone diameter in both of bacteria and Miana leaves extract has strong antibacterial power [18]. It is due to the concentration toward *S. aureus* and *E. coli* have inhibition diameter amounted 10-20 mm.

Miana leaves extract activity in inhibit the growth of bacteria gram negative *E. coli* is more sensitive than bacteria gram positive *S. aureus*. This is due to the differences in the cell wall structure of the two bacteria. The gram positive bacterial cell wall contain of a thick and rigid peptidoglycan layers and teikoat acid. Meanwhile, the gram negative bacteria's cell walls consist of more than one thin peptidoglycan layers and membranes outside the peptidoglycan layer. which is only a few peptidoglycan layers and does not contain teicoic acid, the gram-negative bacteria cell walls were known to be more susceptible to antibiotics or other antibacterial [19].

The compounds which contains of Miana leaves extract also have an important role in providing antibacterial activity. The higher miana leaves extract concentration, hence it will lead clearer the zone diameter. In addition, it shows that the more compounds which have more antibacterial activity, then the inhibition diameter will be better.

The irritation testing result in ointment preparation using Miana leaves extract and

Table 4. The composition of nanoparticles ointment's preparation using the extract of Miana leaves

No.	Materials	Weight in %
1.	Miana leaves extract	1,17
2.	Paraffinum liquidum	10
3.	Propyl paraben	0,2
4.	Methyl paraben	0,1
5.	Vaselin album	100

Table 5. Inhibition Diameter of Miana Leaves Extract with drill diameter amounted 8 mm

Number	Note (%)	Clear zone diameter in <i>S. aureus</i> bacteria (mm)			Clear zone diameter in <i>E. coli</i> bacteria (mm)		
		Petri Dish 1	Petri Dish 2	Average	Petri Dish 1	Petri Dish 2	Average
		1	Extract Concentration 20%	20,00	20,00	20,00	19,30
2	Extract Concentration 40%	22,50	23,00	22,75	24,00	24,50	24,25
3	Extract Concentration 80%	25,50	25,40	25,45	27,70	27,50	27,60
4	Positive Control	30,24	30,48	30,36	31,20	31,60	31,40
5	Negative Control	8,00	8,00	8,00	8,00	8,00	8,00

nanoparticles Miana leaves extract applied in rabbit skin showed that there was no erythema or edema along 3 days observation. Therefore, it can be confirmed that Miana leaves extract ointment and nanoparticles using Miana leaves extract is safe to be applied in skin since it did not cause irritation.

### DISCUSSION

Nanoparticles using Miana leaves extract testing was obtained to ensure that Miana leaves extract powder still have antibacterial activity after drying process. Moreover, this testing also obtained to prove that nanoparticles form more effective since

the administration of medicine is well enough.

Previously, it had been stated that Miana leaves extract amounted 2,5% equal with nanoparticles powder 1,17%.

The result showed that inhibition diameter for nanoparticles powder bigger than the extract in the same concentration. Negative control used was propylene glycol, alcohol 96% and water with the comparison 15:15:15. Afterward, positive control used was chloramphenicol 4 mg/ml ethanol 96%.

### Organoleptic

Organoleptic examination result showed that ointment base or ointment sample which

Table 6. The result of irritation testing

No.	Time after applying (hours)	Rabbit	Extract Ointment		Nanoparticles Ointment	
			Erythema	Edema	Erythema	Edema
1.	1	1	-	-	-	-
		2	-	-	-	-
		3	-	-	-	-
2.	24	1	-	-	-	-
		2	-	-	-	-
		3	-	-	-	-
3.	48	1	-	-	-	-
		2	-	-	-	-
		3	-	-	-	-
4.	72	1	-	-	-	-
		2	-	-	-	-
		3	-	-	-	-

Note :  
 += Irritation  
 -= No Irritation

Table 7. Inhibition diameter result of nanoparticles powder using Miana leaves extract and Miana leaves extract

No.	Note (%)	Pellucid zone diameter in bacteria <i>Escherichia coli</i> (mm)			Pellucid zone diameter in bacteria <i>Staphylococcus aureus</i> (mm)		
		Petri Dish 1	Petri Dish 2	Average	Petri Dish 1	Petri Dish 2	Average
1.	Extract concentration 2,5	15,18	15,32	15,25	14,01	14,05	14,03
2.	Nanoparticles concentration 1,17	16,96	16,89	16,93	14,41	14,45	14,43
3.	Positive control 0,4	31,52	29,49	30,50	31,22	31,15	31,18
4.	Negative control	8,00	8,00	8,00	8,00	8,00	8,00

Table 8. The result evaluation of ointment organoleptic

No.	Ointment	Organoleptic	Week				
			0	1	2	3	4
1.	Base	Color	White	White	White	White	White
		Shape	Semi solid				
		Smell	No aroma				
2.	Nanoparticles using Miana leaves extract	Color	Light brown				
		Shape	Semi solid				
		Smell	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant
3.	Miana leaves extract	Color	Dark brown				
		Shape	Semi solid				
		Smell	Typical smell	Typical smell	Typical smell	Typical smell	Typical smell

Table 9. homogeneity ointment result

No.	Ointment	Weeks				
		0	1	2	3	4
1.	Base	+	+	+	+	+
2.	Nanoparticles using Miana leaves	+	+	+	+	+
3.	Miana leaves extract	+	+	+	+	+

Note : + = homogeneous  
 - = un-homogeneous

Table 10. The result of ointment pH measurement

No.	Ointment	□ pH/ Week				
		0	1	2	3	4
1.	Base	5,28	5,30	5,35	5,39	5,32
2.	Nanoparticles using Miana leaves extract	5,80	5,85	5,90	5,99	5,94
3.	Miana leaves extract	5,49	5,51	5,67	5,75	5,59

Table 11. The result of ointment viscosities measurement

No.	Rpm	Week 0			Week 1		
		Viscosity (cps)			Viscosity (cps)		
		OB	NO	NM	OB	NO	NM
1.	1	73698	85369	76624	74102	85836	77043
2.	2	40802	47263	42421	41025	47521	42653
3.	2,5	40967	47455	42594	41042	47541	42671
4.	4	31593	36596	32847	31651	36663	32907
5.	2,5	36332	42086	37775	36398	42162	37843
7.	2	39128	45325	40682	39199	45407	40756
8.	1	55374	64143	57572	55474	64259	57677

No.	Rpm	Week 2			Week 3			Week 4		
		Viscosity (cps)			Viscosity (cps)			Viscosity (cps)		
		OB	NO	OM	OB	NO	OM	OB	NO	OM
1.	1	74373	87104	79244	75200	88180	80222	75537	88575	80582
2.	2	41402	48489	43871	41631	48817	44169	41931	49169	44487
3.	2,5	41571	48687	43890	41879	49108	44269	42181	49462	44588
4.	4	32059	37546	33847	32297	37871	34140	32529	38144	34386
5.	2,5	36868	43178	38924	37141	43552	39261	37409	43866	39544
6.	2	39705	46501	41920	39999	46904	42282	40288	47242	42587
7.	1	56190	65808	59324	56607	66377	59837	57015	66856	60268

Note: OB: Ointment Base  
 NO: Nanoparticles Ointment using Miana Leaves Extract  
 OM: Ointment using Miana leaves extract

is not contain active ingredient, was white, semi-solid and has no aroma for 5 weeks. Then, nanoparticles ointment using Miana leaves extract has characteristics such as light brown color, semi-solid, and fragrant aroma for 5 weeks. However, it was different with Miana leaves extract ointment which has dark brown color and typical aroma of Miana leaves.

In this case, the nanoparticles ointment using Miana leaves is better if compared with Miana leaves extract. It was because of the fragrant aroma and the color which was not too dark.

### Homogeneity

Homogeneity testing along 5 weeks for the three formulas shows that ointment base, nanoparticles ointment, and Miana leaves extract ointment do not show any change in the homogeneity.

### pH

The pH of ointment preparation expected to be in range of pH 4,5-6,5 so that it will not damage acid coat layer located in the stratum corneum which has function to protect skin from acid and alkali materials. Besides, the acid can suppress the growth of harmful microorganism on the skin.

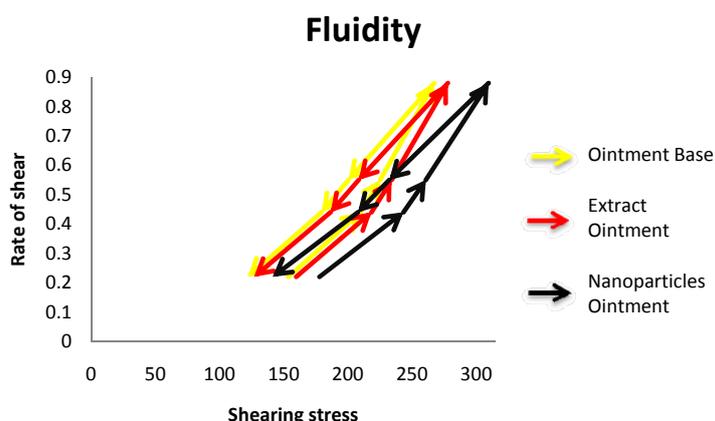


Fig. 1. Ointment Fluidity

Table 12. The result of spread ability

No.	Ointment	□ spread ability (cm)/ week				
		0	1	2	3	4
1.	Base	12,43	12,32	12,11	11,92	12,26
2.	Nanoparticles using Miana leaves extract	10,28	10,11	9,95	9,65	9,80
3.	Miana leaves extract	11,48	11,41	10,79	10,47	11,10

All of the formula was range between 5,28-5,99, hence it still on the expected range which will not damage the acid coat stratum on the skin. The evaluation of the pH between nanoparticles and extract formula were evaluated using Paired sample T-test, since the data were distributed normally with the same samples yet with

different data. The obtained data described that there were difference significant between pH extract and pH nanoparticles extract due to the significance value (2-tailed) was smaller than 0,05 ( $0,000 < 0,05$ ). Therefore, it can be concluded that there were different significant between pH of ointment using Miana leaves extract with the pH of nanoparticles using Miana leaves extract.

#### Viscosity and Fluidity Measurement

##### a. Viscosities

The viscosity assay was performed on rpm 1; 2; 2,5, and 4 using spindle number 25. Generally, each of formula has increasing its viscosity for 4 weeks observation. In the fifth weeks, the preparation has decreasing its viscosity but not too much.

The result of statistical analysis which is using Paired Sample T-test confirmed that there were differences between viscosities of ointment using Miana leaves extract and viscosities of nanoparticles ointment using Miana leaves extract in which the

significant value (2-tailed) 0, 000 smaller than 0,05.

The ointment viscosities which contain of nanoparticles using Miana leaves extract were having the highest viscosities than others. It was due to the smaller particle size will cause the increasing of ointment preparation's viscosities.

##### b. Fluidity

The fluidity assay of all ointment indicate Bingham Bodies, in which the curve does not pass the yield 0,0, but cutting the shearing stress on the yield value. Yield value was depicted as value that should be fulfilled to make the liquid start flowing. In this case, before passing the yield value, the essence was in form of elastically material. But, after passing the yield value, the system flowed based on the newton system in which the shearing stress was proportional to the rate of shear.

#### Spread ability

Spread ability was greatly influenced by viscosity, in which the higher the viscosity, then the smaller the spread ability. In the three ointment preparation showed that ointment with nanoparticle using Miana leaves extract was smaller than the others.

The result of paired sample T-Test showed that there was difference significant between Miana leaves extract and nanoparticles using Miana

leaves extract where the significant value (2-tailed) smaller than 0,05.

## CONCLUSION

1. The minimum inhibitory concentration of Miana leaves' ethanol extract in inhibiting the growth of bacteria *S. aureus* and *E. coli* are in 2,5% concentration.
2. The mixed between Poly Acrylic Acid (PAA) and calcium chloride can change the Miana leaves' ethanol extract to be nanoparticles with the diameter is 89,77 nm using ionic glass method.
3. 2,50% of Miana leaves extract is equal with 1,17% of nanoparticles using Miana leaves extract.
4. The ethanol extract of Miana leaves can be formulated in preparation of hydrocarbon-based ointment with light brown, and fragrant aroma, pH 5,80-5,94, spread ability 9,65-10,28 cm, and has Bingham Bodies characteristics.
5. Nanoparticles using Miana leaves extract provide antibacterial activity better than the extract under the same concentration toward *S. aureus* bacteria (14,43 mm / 14,03 mm) and *E. coli* (16,93 mm / 15, 25 mm) as well as in the ointment preparation toward *S. aureus* bacteria (14,46 mm / 13,58 mm) and *E. coli* (14,59 mm / 13,75 mm).
6. Thus, from irritation testing obtained that nanoparticle of Miana leaves' ethanol extract is safe and do not cause irritation.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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